

In re: Giritch et al.
International Appl. No.: PCT/EP2004/000892
International Filing Date: January 30, 2004
Amdt. Dated 02/04/2008

Amendments to the Specification:

Please add to the specification the Sequence Listing filed concurrently herewith using the EFS-Web Electronic Filing System.

Please replace the second paragraph that begins on page 20 with the following paragraph.

Fig. 17 depicts schematically vectors pICH13630, pICH15760 in (A), and pICH10881, pICH15770 in (B). The adapt3 and adapt4 adapters shown in (A) correspond to SEQ ID NOS: 7 and 8, respectively.

Please replace on page 31 the paragraph that begins immediately below the heading "*Design of pICH11150*" with the following paragraph.

This construct was done on the basis of binary vector pICBV-19 (Figure 2). As a first step of cloning, the target Bsal restriction sites for the intron insertion were introduced into the BAR gene (construct pICH10605, Figure 2). The Bsal enzyme cuts DNA outside of the recognition site making 4 nucleotides overhang. In the case of pICH10605, the Bsal enzyme was used to introduce splicing acceptor and donor sites for the consequent intron insertion. As a next step, PCR fragment amplified on pICH7410 (Figure 3) construct with oligos int-ad-9 (5'-ttttggtc cgacctgcaa caataagaac aaaaagtcat aaatt-3'; SEQ ID NO: 1) and attbpr11 (5'-ttaagctt agctttcc taggtcgaa gccgcggc gggtg-3'; SEQ ID NO: 2) was inserted into pICH10605 using Bsal and HindIII restriction sites. The PCR fragment containing AttB and 3' part of intron as well as AvrII and SacI restriction sites replaced the GUS expression cassette and 5'part of

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BAR expression cassette. The T-DNA part of the resulting construct (pICH11140, Figure 4) contained the 3' part of BAR expression cassette: AttB, 3'part of the intron, 3' part of BAR-gene and OCS terminator as well as AvrII and SacI restriction sites. As a final step of 3' construct cloning, a PhiC31 integrase expression cassette containing *Arabidopsis* actin 2 promoter, PhiC31 integrase and NOS terminator was introduced into pICH11140 using AvrII and SacI restriction sites. The final construct pICH11150, containing 3' end of BAR gene with AttB, recombination site together with the 3' end of the intron, as well as PhiC31 integrase expression cassette is shown in Figure 4.

Please replace the paragraph that bridges pages 31-32 with the following paragraph.

This construct was done on the basis of binary vector pICBV-16 (Figure 5). The PCR fragment amplified from pICH8430 (Figure 5) with oligos int-ad-10 (5'-tttaagcttg aattctttg gtctcaggta agtttcattt tcataattac aca-3'; SEQ ID NO: 3) and attppr14 (5'-ttttcaatt ggagctccta cgcccccAAC tgagagaac-3'; SEQ ID NO: 4) was cut with HindIII and MfeI restriction enzymes and introduced into pICBV-16 digested with HindIII and EcoRI. PCR fragment containing 5' part of intron and AttP as well as BsaI and EcoRI restriction sites replaced the GUS expression cassette in intermediate construct pICH11160 (Figure 6). As the final step of the cloning, EcoRI/BsaI fragment of pICH10605 (Fig. 2) containing a NOS promoter and 5' part of BAR gene was inserted into pICH11160. The T-DNA region of the final construct pICH11170 is shown in Figure 6.

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Please replace on page 32 the paragraph that begins immediately below the heading "*Design of pICH15850*" with the following paragraph.

The NotI/SacI fragment of pICH11170 (Fig. 6) was fused with adapters adipt1 (5' ggccgc~~tttt~~ tatgcattt ttgagctct cgcgaggatc ctagct 3'; SEQ ID NO: 5) and adipt2 (5' aggatcctcg cgagagctca aaaaatgcat aaaaagc 3'; SEQ ID NO: 6) that destroyed the original SacI site and introduced BamHI, SacI and NsI sites, producing pICH15830 (Fig. 16). For pICH15840 cloning, the NotI/NsI fragment of pICBV2 (Fig.16) was transferred to the pICH15830 (Fig.16) construct, reintroducing T-DNA left border region which was excised in the first step of cloning. The BamHI/SacI fragment of pICH15820 (Fig. 14) containing complete IPT gene was transferred to pICH15840, resulting in pICH15850 (Fig. 14).